PRO4000X a tool for improving water quality and reducing stress

Stephen G. Newman Ph.D.
President and CEO
AquaInTech Inc.

How our products can help

Cleaner Pond Bottoms =

- Complete reduction of ammonia
- Shorter time between cycles
- Less lime needed to oxidize organic material
- Lower levels of hydrogen sulfide
- Less water exchange required

Increased Survivals

Better Growth

- Less stress
- Lower levels of vibrios
- Less blue green algae
- Eliminate antibiotics

Excellent cost benefit due to reduced costs of production as a result of improved quality of environment

Stress the biggest problem aquaculturists face

- What is stress?
  - Production of animals in environments that are not ideally suited for optimum animal physiological functioning is the norm for most shrimp aquaculture rather than the exception
  - Result is that there is less then ideal growth, increased susceptibility to diseases, pathogen and environment related and less productivity with overall consequences that result in LOWER PROFITS

What can you do to lessen the stress?

- Improve culture conditions to create a healthier environment
- Increase dissolved oxygen levels, lessen the build up of metabolites such as ammonia and hydrogen sulfide, reduce the organic matter loads to lower nutrient loads that contribute to eutrophication
- Some things are inherent in the production system, others can be readily controlled
- Our products are tools-part of a solution.

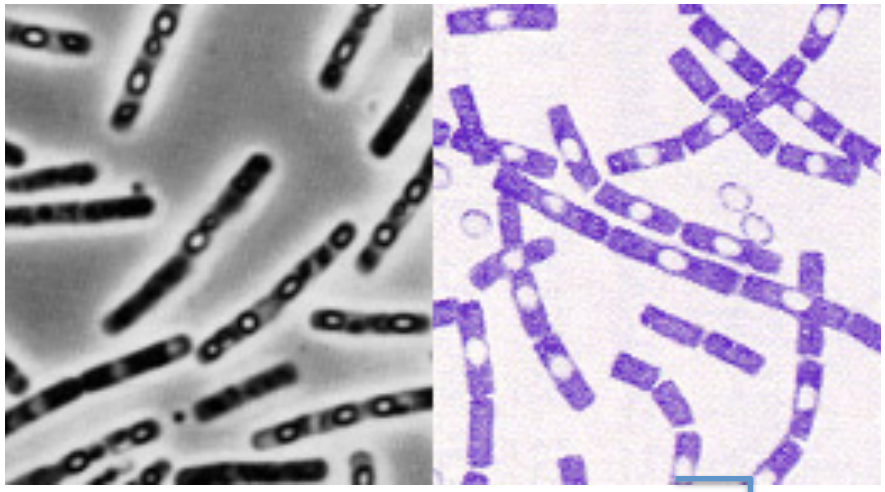
Tools for lessening the stress

- PRO4000x family of products
- Bacillus based
  - Natural strains selected for specific biochemical properties
  - Stable spores indefinite shelf life when kept free of moisture
  - Field proven
  - Targeted delivery for common sense application

How do the tablets work?

- Each tablet contains more than 60,000,000,000 spores of two of our selected bacterial strains.
- The tablets are thrown into ponds where they sink to the bottom and dissolve
- As they dissolve the spores germinate (fancy word for hatch) and proliferate if the environment is favorable

The Bacteria

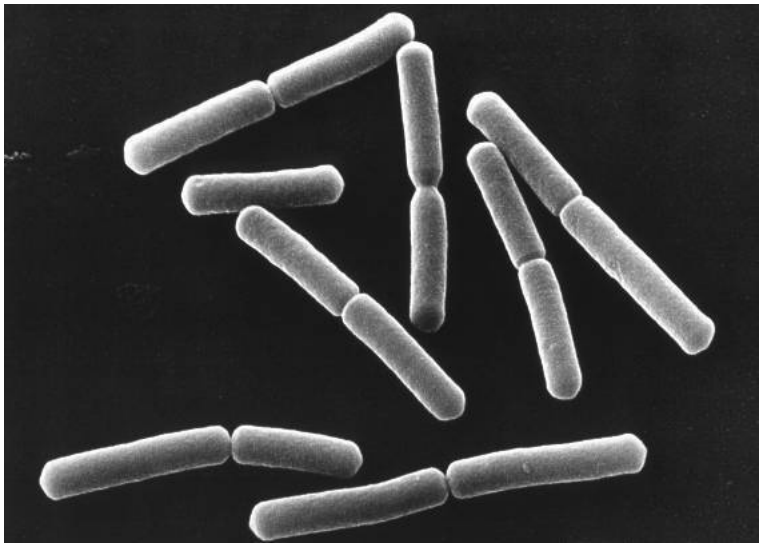


Stable spore forming bacteria. The spores allow the bacteria to survive in environments that kill other bacteria.

Bacillus subtilis

- Natural occurring in soils 10^6 spores per gram
- Human digestive tract 10^4 spores per gram
- Considered to be a normal inhabitant of many animals digestive tracts
- Very large and diverse group of bacteria many different strains

Electron microscope photo



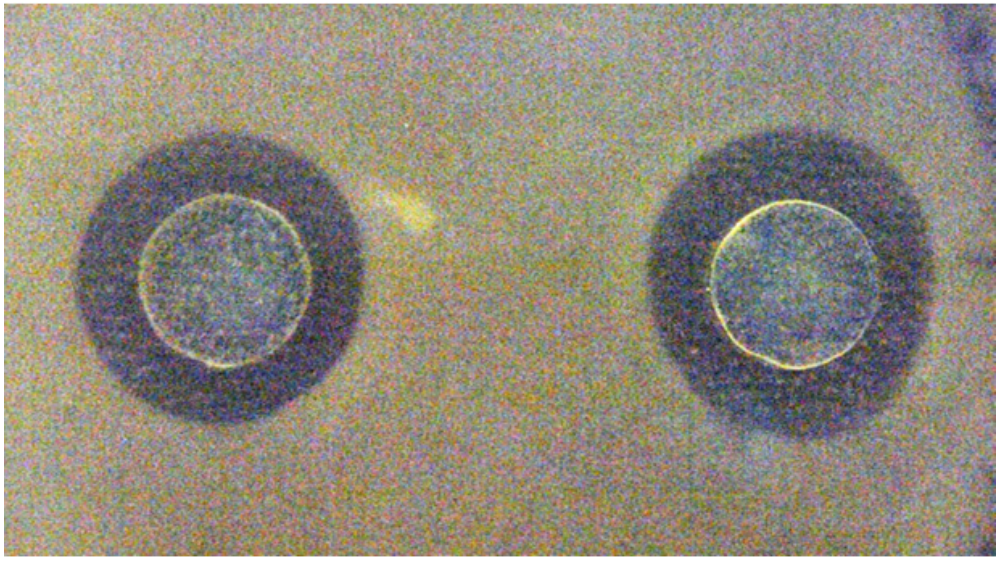
Colonies on agar

Bacillus licheniformis

- Produces a variety of enzymes involved in the recycling of organic matter and other nutrients.
- Very tolerant of environmental extremes

Modes of action

- Direct inhibition by the production of bacteriocins.
- Picture below shows inhibition of a vibrio strain (clear zone) by our *Bacillus subtilis*.



Modes of action

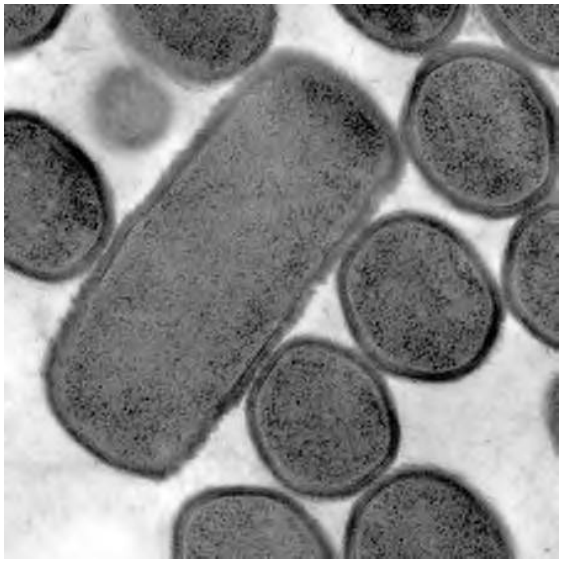
- Indirect inhibition by the consumption of nutrients. Bacteria will compete against other bacteria and algae in the ponds.

Before

After

Miscellaneous Bacillus species

- Form a complex that working together optimizes the impact of the tablets
- Several different strains with different properties



Mode of action

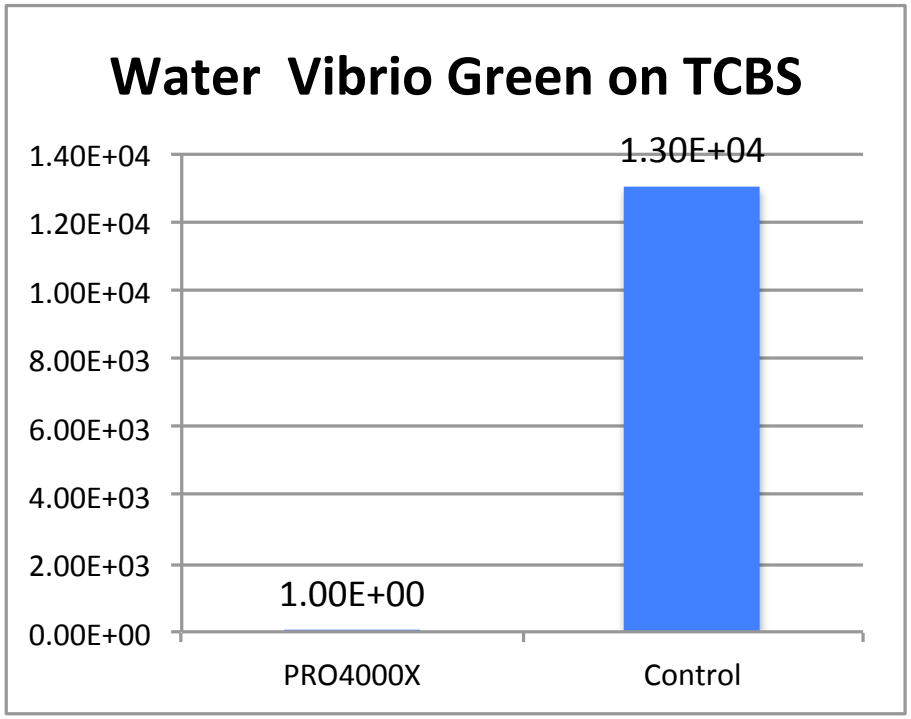
PRO4000X tablets

What is the best way to use PRO4000X?

- Each environment is unique. Ponds are never the same. Differences include biomass, native bacterial populations, waste products from shrimp culture (nitrogen in the form of ammonia and fecal material), uneaten feed, etc.
- Optimum usage determined by trial and error
- Guidelines provided
- As the farmer you know how this product will work the best for you

Hatchery studies

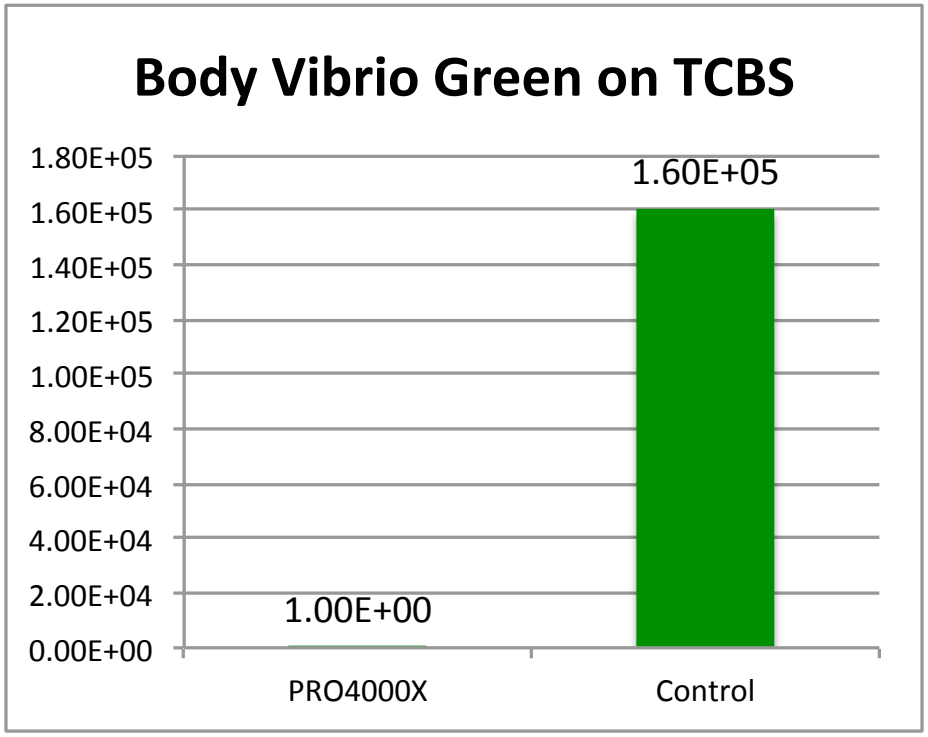
- Reduction in harmful vibrio (s) in the water



Potentially pathogenic vibrios reduced by 13000 fold (>99.99%)

Hatchery studies

- Reduction in harmful vibrio (s) on the animal



Potentially pathogenic vibrios reduced by 160000 fold (>99.999%)

- Every pond is different
- Protocols will vary between ponds and farms
- Products that offer set protocols fail to take this into account
- Targeted delivery to problem areas of selected bacteria is a common sense approach
- Pouring liquids and powders into ponds cannot have the same impact-physics determines this

- Start out with low levels of tablets
- Distribute tablets
  - Problems areas of the ponds (corners)
  - Drainage canals (both inside the ponds and outside canals)
  - Sedimentation ponds
- Add a source of carbon if possible, such as molasses to enhance bacterial growth

Suggested usage for more than 70 animals per m2

Day #	Tablets	Cumulative
7	10	10
14	25	35
21	35	70
28	45	115
35	60	175
42	75	250
49	90	340
56	100	440
63	110	550
70	120	670
77	130	800
84	140	940
91	140	1080

Start out slow and gradually increase the number of tablets. Focus on known problem areas based on pond history, exits and inlets and and drainage areas where sludge can accumulate. Add remainder to pond bottoms.

Add Molasses or another suitable carbon source-it helps the bacteria grow. You can soak the tablets in it or just add it to the pond at 10 to 20 liters per ha per application.

Suggested usage for less than 70 animals per m2

Day #	Tablets	Cumulative
7	10	10
14		
21	15	25
28		
35	25	50
42		
49	35	85
56		
63	50	135
70		
77	75	210
84		
91	90	300
98		
105	100	400
112		
119	110	510
126		
133	110	620
140		

Start out slow and gradually increase the number of tablets. Focus on known problem areas based on pond history, exits and inlets and and drainage areas where sludge can accumulate. Add remainder to pond bottoms.

Add Molasses or another suitable carbon source-it helps the bacteria grow. You can soak the tablets in it or just add it the pond at 10 to 20 liters per ha per application.

Who can use PRO4000X?

Shrimp farms, semi intensive

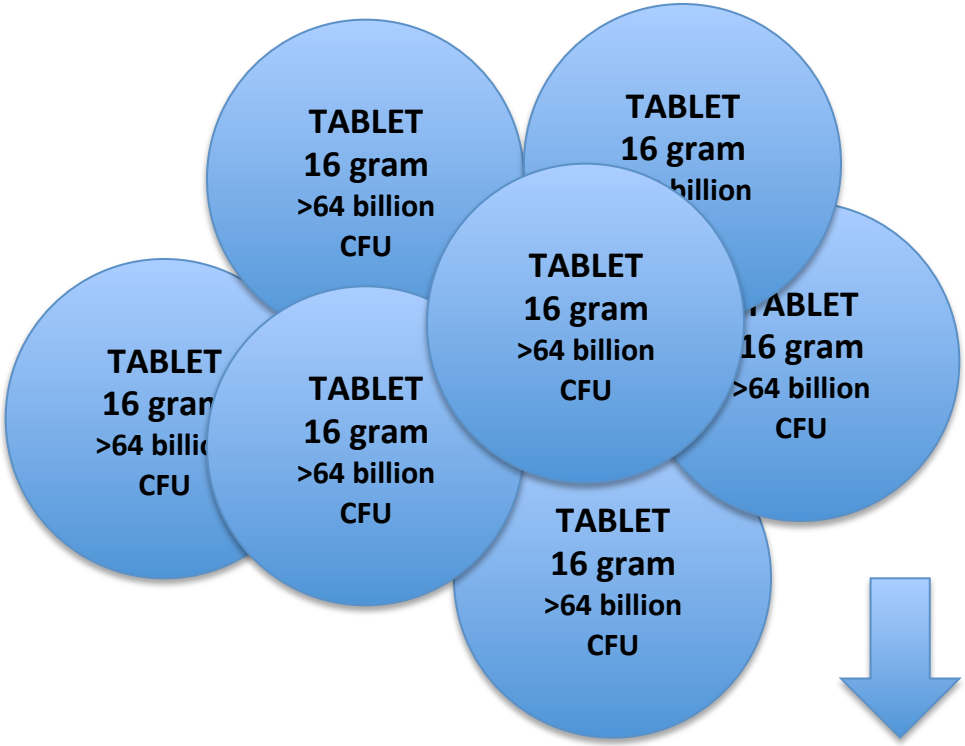
Royal Mayan Shrimp Farm, Belize

Intensive shrimp farms

Vietnam

Tilapia Nursery Indonesia

This versatile product works in farms, any pond, lake, reservoir or other similar body of water, golf course ponds, koi ponds, hatchery tanks, etc.



Degradation of organic matter resulting in improvement of water quality.

Used properly can compete against other bacteria (vibrios) and blue green algae. Note that the impact that you see will depend on your application rates and your environment

Bacteria alter water composition

Before

After

Note the color difference

Other products for other applications

Biodegradable bags with a minimum of one trillion (1,000,000,000,000) CFU per bag. Use in stubborn areas.

Requires water to be calm. Bags settle to the bottom and dissolve with nutrients and spores

Can be easily incorporated into feed. Bags can be added during the milling process. They dissolved and the components are mixed with the feed. One bag per MT results in approximately 106 spores per gram of feed. The spores do not germinate in the shrimp guts as the transport time is too short, although it is known that in the hepatopancreas of the shrimp residence times can be days. After the shrimp defecate the spores germinate in the feces. They are readily re-ingested as shrimp forage. It is not likely that there is anything but a short term colonization as shrimp do not have stable microbial flora.

Conclusions

- Targeted delivery of tablets to pond bottoms ensures bacteria are in the problem areas
- Bacteria will grow and degrade the nutrients immediately present and spread out from sediments into water column until nutrients become limiting and they die off.
- Adding more bacteria and carbon ensures optimum performance
- Clients have reported a variety of impacts
- How many tablets and how often to apply them is derived from experience and the provided guidelines